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THE EFFECT OF LIGHT ON OPTICAL DENSITY OF PLANT PIGMENT EXTRACTS OF FECES IN DIGESTIBILITY STUDIES[1/]

by

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In connection with digestibility studies using ratio technique, Lancaster and Bartrum have reported that acetone plant pigment extracts of feces increase in optical density on exposure to light.

In connection with this finding, the following experiments have been conducted. This laboratory has found that acetone fecal extracts are remarkedly sensitive to light, which produces serious increases in optical density readings.

Fecal samples were taken with precautions to avoid exposure of light. Samples were collected immediately after passage, and extracted with 85% acetone. Extractions were carried out in a room with lights off and shades down. The Waring blendor used in extractions was equipped with an aluminum blending jar. When extraction was complete, the extracts were diluted and read in a Beckman DU spectrophotometer at wavelengths 406 and 415 mμ. The solutions were then exposed for one hour to daylight. The intensity of the daylight was regulated to read 4 on a Weston exposure meter, model 650. A Weston reading of 4 is equivalent to 16 foot candles.

The percentage increases in optical density of fecal extracts due to light exposure are shown in Table 1. It is noted that greater increases are obtained at 415 mμ than 406 mμ.

Since the fecal extracts from a calf maintained on synthetic milk exhibited this light sensitivity, it might indicate that the pigments involved in this reaction may originate in the bovine digestive tract.

As bile is known to contain tetre-pyrrolic compounds similar to the degradation products of chlorophyll, samples of bile obtained from slaughtered cattle were extracted with 85% acetone and placed with acetone extracts of Kentucky bluegrass. Light reaction was negative for this combination. In the dark an increase in optical density was observed due to the interaction of bile and Kentucky bluegrass extracts.

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Table 1.-The percentage increase in optical density of acetone fecal extracts after exposure to light for one hour.

	Increase 406 mμ %	Increase 415 mμ %
Orchard grass Ladino clover	19.1	29.1
Bluegrass	37.6	52.1
Orchard grass, clover and bluegrass	31.1	45.6
Orchard grass 1954	6.2	7.0
Orchard grass 1955	9.6	14.7
Synthetic milk	39.5	53.4

In Table 2 the effect of exposing feces to light prior to extraction, and the effect of subsequent light exposure of the fecal acetone extracts are shown. Data indicate that light has a direct effect on the feces itself, as well as on the acetone extracts. Feces samples were spread in a thin film on paper and aluminum foil and exposed to daylight before a south window.

Table 2.-Effect of light on feces (Date in optical densities of acetone fecal extracts per gram of dry feces).

	Dark	1 hr. light	% Increase
406 mμ			
Feces Control - No light	1117	1537	38
Feces after 7 hours light	1470	1637	11
7-hour light % increase	32	11	
415 mμ			
Feces Control - No light	1317	1983	50
Feces after 7 hours light	1961	2256	12
7-hour light % increase	49	11	

The effect of autoclaving the feces before extraction is shown in Table 3. The extracts from feces samples that had been autoclaved 75 minutes changed little when exposed to light. In other words, autoclaving for 75 minutes inhibits light effect on acetone extracts of the autoclaved material. Autoclaving the feces for 15 or 30 minutes produces increases in optical densities of the resulting acetone extracts.

Table 3.-Effect of autoclaving feces (Data expressed in E-values of acetone fecal extracts per gram dry matter.

Time in autoclave	406 mμ			415 mμ		
	Dark	1 hr. light	% Increase	Dark	1 hr. light	% Increase
Control	1117	1537	38	1317	1983	50
15 minutes	1228	1498	22	1591	1990	25
30 minutes	1238	1487	20	1602	1968	23
75 minutes	1189	1224	3	1579	1637	4

Heating feces in a drying oven for 6 hours at a temperature of 80° C eliminates the optical density gain on exposure to light. However, it was found that although no light increase was noted, solutions of feces that had been heated agreed in spectrophotometric readings with solutions exposed to light, and not with the exposed control samples. In this case heat energy appears to produce the same effect as light energy.

Various chemical reagents were added to acetone fecal extracts to ascertain their effect on light reaction of acetone fecal extracts. The presence of ascorbic acid or oxalic acids intensified the increase in optical density upon exposure to light. Hydrogen peroxide, hydroquinone, perchloric acid had no apparent effect on the light reaction.

Conclusions:

1. Exposure of acetone fecal extracts to light increases the optical density of solutions at wavelengths 406 mμ (38%) and 415 mμ (50%).

2. Light exposure of feces prior to acetone extraction produces increases in optical density over feces samples kept in dark.

3. Feces autoclaved for 75 minutes at 15 lbs. pressure differed insignificantly after light exposure from unexposed control samples when read at wavelengths of 406 mμ and 415 mμ.